fused to Rem core/carboxyl-terminus inhibited L-current with a concurrent reduction in charge movement. Thus, our work demonstrates that Rad and Rem exert their inhibitory effects on L-type channels in differentiated muscle fibers via distinct mechanisms. We have identified the amino-termini of Rad and Rem as the structural elements which dictate these specific modes of inhibition of $Ca_V1.1$. Supported by AG038778 (RAB) and 2T32AG000279-11 (Schwartz).

700-Pos Board B455

Pegylated-Cholesterol Decreases the Amplitude and Augments Time- and Voltage-Dependent Inactivation of L-Type CA^{2+} Current of A7R5 Cells from Rat Aorta

Rikuo Ochi[1,2], Sukrutha Chettimada[1,3], Sachin A. Gupte[1,2].
[1]University of South Alabama, Mobile, AL, USA, [2]New York Medical College, Valhalla, NY, USA, [3]Harvard University, Boston, MA, USA.

Cholesterol (Chol) localizes at high density in lipid rafts and caveolae in the membrane and regulates ion channel functions. We studied Chol-dependent regulation of L-type Ca^{2+} channel current ($I_{Ca,L}$) by applying water- and lipid-soluble pegylated Chol. A7r5 cells, a cell line from fetal rat aorta, were incubated with PEG600:Chol (PC) for hours in microtubes. PC dose-dependently (0.1 - 10 mM) decreased the density of $I_{Ca,L}$ recorded by whole-cell clamp method with Ba^{2+} as a charge carrier to 51%, while similar pretreatment by methyl-beta-cyclodextrin (MbCD) dose-dependently (1 - 30 mM) increased it by 61%. Voltage-dependence of the activation was little affected by them. PC accelerated the time course of the current decay: the ratio of the amplitude at 500 ms to the peak amplitude (I_{500}/I_{peak}) at 0 mV was decreased from 0.41 in control to 0.26 by 1 mM PC, while the pretreatment by MbCD increased it to 0.63. The PC-pretreatment shifted f_{inf}-V curve to the left, shifting $V_{0.5}$ from control −29.5 mV to -39.2 mV at 1 mM. In contrast, MbCD shifted $V_{0.5}$ to the right to −25.5 mV at 30 mM. The PC-induced decrease of $I_{Ca,L}$ density was reversed to an increase by the addition of 30 mM-MbCD to the tube containing 1 mM-PC. However, it only moderately reversed the PC-induced decrease of I_{500}/I_{peak} and hyperpolarizing shift of f_{inf}-V. By inhibiting amplitude and augmenting inactivation, PC strongly inhibited window $I_{Ca,L}$ that is mainly responsible for depolarization-induced contraction in the arterial smooth muscles. PC-induced increase of membrane stiffness due to the increase of the total content of Chol and/or direct interaction of PC with signaling lipids and proteins including $Ca_V1.2$ could explain these changes of $I_{Ca,L}$.

701-Pos Board B456

AKAP79/150-Anchored CaN and PKA Regulate Neuronal L-Type Calcium Channel Activity and NFAT Transcriptional Signaling

Jonathan G. Murphy, Mark L. Dell'Acqua.
Dept. of Pharmacology and Neuroscience Graduate Program, University of Colorado, Anschutz Medical Campus, Aurora, CO, USA.

In neurons, Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels (LTCC) couples electrical activity to changes in transcription. LTCC activity is elevated by the cAMP-dependent protein kinase (PKA) and depressed by the Ca^{2+}-dependent phosphatase calcineurin (CaN), with both enzymes localized to the channel by A-kinase anchoring protein (AKAP) 79/150. AKAP79/150 anchoring of CaN also promotes LTCC activation of transcription through dephosphorylation of the nuclear factor of activated T-cells (NFAT). We report here that genetic disruption of PKA anchoring to AKAP79/150 also interferes with LTCC activation of CaN-NFAT signaling in neurons. Disruption of AKAP-PKA anchoring promoted redistribution of the kinase out of dendritic spines, profound decreases in LTCC phosphorylation and Ca^{2+} influx, and impaired NFAT movement to the nucleus and activation of transcription. Our findings support a model wherein basal activity of AKAP79/150-anchored PKA opposes CaN to preserve LTCC phosphorylation, thereby sustaining LTCC activation of CaN-NFAT signaling to the neuronal nucleus.

Voltage-gated K Channels I

702-Pos Board B457

High Yield Expression of the Human *Ether-à-Go-Go* Related Gene (HERG) in *Saccharomyces Cerevisiae*

Karen Molbaek[1], Peter Scharff-Poulsen[2], Dan A. Klaerke[1], Per Amstrup Pedersen[2].
[1]Dept. of Physiology and Biochemistry, University of Copenhagen, Frederiksberg, Denmark, [2]Dept. of Biology, University of Copenhagen, Copenhagen, Denmark.

The human *Ether-à-go-go* related gene (hERG) encodes a voltage-gated potassium channel and represents the molecular correlate of the IKr current, which is one of the potassium currents involved in the repolarizing of the cardiac action potential. Inhibition of hERG prolongs the QT interval in the electrocardiogram and enhances the risk for severe or even fatal arrhythmias. Detection of unintended interactions with hERG is therefore an important issue when compounds are approved for drug development. In the present study we have explored *Saccharomyces cerevisae* as a host for heterologous expression of the hERG channel. Yeast codon optimized hERG cDNA was used to generate expression plasmids producing the hERG channel C-terminally fused with either a His10 or a TEV-GFP-His8 tag. The latter was generated to ease quantification of the expression level, to allow live cell bioimaging, development of a purification protocol and assessment of the quality of the recombinant protein. Both gene fusions were expressed from a galactose inducible promoter located on a plasmid with a regulatable copy number in a yeast strain overexpressing the Gal4 transcriptional activator. 48 hours after induction recombinant hERG accumulated to approximately 1.6% of total membrane content when produced at 15°C in amino acid complemented media. A solubilization screen established Fos-Choline-12 as a superior detergent for hERG solubilization. Solubilization in Fos-Choline-12 supplemented with cholesterol hemisuccinate generated a monodisperse FSEC (fluorescent size-exclusion chromatography) profile and caused recombinant hERG to elute in its native tetrameric form. In-gel fluorescence of SDS-separated yeast membranes showed that recombinant hERG protein has the expected molecular weight. Complementation assays in *S. cerevisiae* revealed that the heterologuosly expressed hERG is able to rescue the high potassium requirement of a *trk1*Δ, *trk2*Δ yeast strain indicating that the recombinant hERG is functional.

703-Pos Board B458

Expression and Purification of a Functional hERG Pore Domain for Biophysical and Electrophysiological Studies

Maïwenn Beaugrand[1], Sumit Kalsi[2], Andrée E Gravel[1], Christopher D. Johnson[3], Neville Wright[3], Jason C Young[4], Maurits R.R. de Planque[2], Isabelle Marcotte[1], Philip T.F. Williamson[3].
[1]Département de Chimie, Université du Québec à Montréal; GRASP/CQMF, Montréal, QC, Canada, [2]Electronics and Computer Sciences, Institute for Life Sciences, Southampton, United Kingdom, [3]Centre for Biological Sciences, Institute for Life Sciences, Southampton, United Kingdom, [4]Department of Biochemistry, McGill University, Montréal, QC, Canada.

The long QT syndrome (LQTS) is a cardiac dysfunction that prolongs the heart repolarisation interval, leading to cardiac arrhythmia or failure. This pathology can be induced by off-target effects of drugs which block the human ether-a-go-go related gene (hERG) potassium channels located in the myocardium cell membranes. To reduce the risks of this acquired LQTS, regulatory authorities demand in vitro testing of all new drug entities for hERG-blocking potential. As the pore domain of the hERG channel is an important target of LQTS-prone drugs, the objective of our work was to express and purify this region (Asp540-Tyr673) in E. coli to allow biophysical and electrophysiological studies. The detergent sarkosyl was employed for the solubilisation and a His6 N-terminal tag was used to isolate the transmembrane pore domain of hERG with yields of approximately 0.5-1 mg per liter of LB media. Mass spectrometry and Western blot confirmed the identity of the protein and circular dichroism showed that the majority of the hERG pore domain segments adopt an α-helix structure as was expected from sequence homology with other K^+ channels. The functionality of the channel is proven by incorporating it into lipid bilayers formed using Montal Mueller method.

704-Pos Board B459

Eag Domains Regulate LQT Mutant hERG Channels in Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Qiangni Liu, Matthew C. Trudeau.
University of Maryland, Baltimore, Baltimore, MD, USA.

The generation of human induced pluripotent stem cells (hiPSCs) by controlled delivery of reprogramming factors provides a novel path for a wide range of disease research, including type 2 long QT syndrome (LQT2). The human ether-a-go-go related gene (hERG) encodes the α-subunit of a voltage-gated potassium channel underlying IKr. hERG potassium channels contain nearly 300 different disease-causing mutations, which can lead to LQT2. Its N-terminal region contains an eag domain, which is important for modulating channel deactivation properties. R56Q is a LQT2-associated point mutation located in the eag domain, which is a defect known to increase the rate of deactivation profoundly. We previously showed that isolated eag (i-eag) domains rescued the dysfunction of hERG R56Q channels by replacing the covalently attached, but defective eag domains when the channels were expressed in Xenopus oocytes or HEK 293 cells. Our goal was to determine whether the rescue of